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Effect of tear supplements on signs, symptoms and inflammatory markers in dry eye

Eilidh Martin[a,*], Katherine M. Oliver[a], E. Ian Pearce[a], Alan Tomlinson[a], Peter Simmons[b], Suzanne Hagan[a]

[a] Vision Sciences, Glasgow Caledonian University, Glasgow, Scotland, United Kingdom
[b] Allergan plc, Irvine, CA, USA

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ABSTRACT

Purpose: Three tear supplements were compared for their effects on the signs, symptoms and inflammatory status of subjects with dry eye disease. Assessments were made before and after both 2 and 4 weeks of treatment.
Methods: In this masked, randomized, 3-way crossover trial, eighteen dry eye subjects were recruited. At each visit, symptoms, tear evaporation rate, stability and osmolarity were measured and tear samples were analyzed for 7 inflammatory markers, using multiplex immunoassays. The 3 treatments included carboxymethylcellulose-glycerine-castor oil (CGC), carboxymethylcellulose (CMC) and hydroxypropyl guar (HPG). The CGC and HPG drops are emulsified lipids; CGC also contains osmoprotectants. The CMC drop is a standard aqueous polymeric supplement.
Results: Significant improvements were seen in symptoms (OSDI) and tear stability (NITBUT) with all 3 treatments at 4 weeks. At 4 weeks post-CGC, 6 out of 7 biomarkers demonstrated a > 25% reduction (in 40% of subjects). The same reduction (> 25%) was seen in 10% of the subjects for CMC and in none of the subjects for HPG. No significantly different change to either evaporation rate or tear osmolarity was found following any of the three treatments.
Conclusions: In this study, the CGC treatment resulted in the greatest reduction in ocular biomarkers of inflammation, while all 3 treatments reduced symptoms and improved tear stability. These results indicate that subject-perceived symptomatic improvements are not necessarily associated with a reduction in objective measures of inflammation.

1. Introduction

Dry eye disease (DED) is a common ocular condition that presents with a wide range of signs and symptoms. It has an estimated prevalence of between 5 and 30%, depending on the study population [1–6]. The recent International Dry Eye Workshop (DEWS II 2017) has established that DED may be caused by numerous factors, including age, hormonal changes (especially in females), eyelid conditions, systemic conditions and a variety of environmental influences, including adverse environments and contact lens wear [7–9]. Regardless of etiology, DED may involve ocular surface inflammation and a loss of the homeostasis of the tear film [8,10–12]. Research has shown that evaporative water loss to leading to tear hyperosmolarity is the primary cause of the patient's discomfort, tissue damage and the inflammatory response [7,13–15]. Tear hyperosmolarity leads to the secretion of inflammatory mediators at the ocular surface and detected in the tears [16,17]. These mediators, in turn, lead to ocular surface damage, i.e. epithelial cell damage [18].

There is, currently, no "gold-standard" test available for this condition and existing diagnostic tests show poor agreement with patient symptoms when diagnosing DED [19–25].

Inflammatory biomarker analysis of tear fluids has emerged as a novel method of quantifying the inflammatory response in DED, with research demonstrating an increase in the concentration of inflammatory protein biomarkers (cytokines and chemokines) in patients with DED [26–28]. These inflammatory proteins have also been shown to be related to the signs and symptoms of DED [29,30]. Inflammatory biomarkers can also be associated with adverse environmental conditions and various inflammatory pathologies, where they have been proposed as diagnostic markers in allergic eye disease, graft versus host disease and keratoconus [31–35]. In addition, using cytokine levels as a measure of the inflammation associated with DED has the advantage of

* Corresponding author at: Vision Sciences, Glasgow Caledonian University, Glasgow, Scotland G4 0BA, United Kingdom.
E-mail address: eilidh.martin@gcu.ac.uk (E. Martin).

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being objective. These measures could be used in assessing DED severity, monitoring disease progression and in quantifying treatment effects [36–38].

As a result of recent advances in the sensitivity of commercial protein assays (e.g. Multiplex bead arrays), up to 27 biomarkers can be assessed at picograms per milliliter (pg/ml) levels. Consequently, analysis can be made of tear samples that are only 1 µl in size [39,40]. However, this technique can be limited by the difficulty in collecting even, 1 µl, tear samples from DED patients. Furthermore, inter-subject variability has so-far prevented the establishment of clear cut-off levels for a positive disease state [37].

While some DED patients may receive anti-inflammatory treatment, such as steroids or cyclosporine, almost all patients (regardless of the level of severity) use one or more artificial tear formulations, primarily to manage symptoms. Numerous clinical trials have reported relief of symptoms and a reduction in clinical signs with the use of artificial tears [41–43]. For example, a report compared the effect of two artificial tear formulations on matrix metalloproteinase-9 (MMP-9) and IL-6 levels [44]. However, broader clinical investigations of the potential effects of artificial tear formulations on inflammatory biomarkers have not been reported.

Tear supplements use a variety of different ingredients with the aim of compensating for tear film deficiencies in dry eye. These include soluble polymers (to provide hydration and lubrication), beneficial electrolytes (essential to the physiological function of the cornea) and emulsified lipids (to reduce evaporation of the aqueous component of the tear film). Some formulations also contain small non-electrolytes, such as polyols or amino acids. These can function as osmoprotectants to provide protection from the cellular stress caused by hyperosmolarity, which is characteristic of dry eye [45,46].

In this study, 2 formulations containing emulsified lipids, one of which also containing osmoprotectants, were compared to a standard aqueous polymer formulation. The intention was to compare the effects of lipid supplementation alone, lipid supplementation plus protective non-electrolytes and a standard polymeric aqueous formulation, on inflammatory biomarkers, as well as on the signs and symptoms of DED.

2. Materials and methods

Recruitment for this study was through email and poster advertisements within Glasgow Caledonian University (GCU) and all tests were carried out at GCU. The targeted group was those with evaporative dry eye (EDE), in order to investigate the potential benefit for the lipid-based formulations under study. Eighteen (of the 21 recruited) dry eye subjects (14 female, 4 male and mean age 30 ± 14 years) completed participation in this study. Dry eye status was confirmed with a non-invasive tear break-up time (NITBUT) of < 10 s and an Ocular Surface Disease Index (OSDI) score of > 12. Moreover, when the study commenced, all subjects had an elevated tear evaporation rate (TER) f > 33 $g/m^{-2}/h$ [47]. A Schirmer test was also carried out at the screening visit to establish tear production levels (see Table 1 for screening visit data). The Schirmer test was performed without anesthetic, with the strip placed at the lateral third of the lid margin for 5 min. Throughout the study, the more subjectively-symptomatic eye was investigated or, if there was no perceived difference, then the right eye was examined. Exclusion criteria included: active ocular allergy; current contact lens wear; use of any topical ophthalmic drops within 1 week of the screening visit and commencement of the study (initial wash-out period); ocular surgery within the last 12 months; change/addition to any chronic systemic medication known to affect tear production (including antihistamines, antidepressants, diuretics and corticosteroids) within 30 days of any visit; systemic disease known to affect tear production or loss that had been diagnosed or had not been stable within 30 days of visit.

This was a randomized, repeated-measures study; each subject received all 3 treatments over a 14 week period, as shown in Fig. 1. It was a single-blind study, whereby subjects were masked as to which treatment they were receiving. Subjects were instructed to instill the eye drops into both eyes and not to use the drops within two hours of each assessment visit. Subjects were instructed to use the drops 4 times each day, and given a checklist to ensure compliance.

Table 1
Demographic and screening visit data of subjects detailing age, sex, drops used prior to the study commencing, OSDI score, NITBUT (average of 3 measurements in seconds) and Schirmer (mm of wetting in 5 min).

Subject	Age	Sex	Drop use prior to study	OSDI	NITBUT	Schirmer
1	21	M	Guar Gel	18.2	5	27
2	53	F	None	33.3	7	0
3	42	M	Carmellose + glycerine	16.7	7	4
4	27	F	Sodium hyaluronate	15.0	4	5
5	42	F	None	14.6	6	2
6	25	F	Sodium hyaluronate	22.9	8	28
7	26	F	Hypromellose	22.9	7	20
8	21	F	None	18.2	8	28
9	58	F	Hypromellose and carbomer	12.5	5	7
10	22	F	Sodium hyaluronate	18.2	5	12
11	21	F	Hypromellose	20.5	5	7
12	60	M	Carmellose + glycerine	13.6	6	11
13	23	F	Carbomer	34.1	8	14
14	20	F	Sodium hyaluronate	16.7	7	5
15	20	M	None	16.7	6	5
16	20	F	Hypromellose	14.6	5	22
17	23	F	Sodium hyaluronate	33.3	6	13
18	20	F	Sodium hyaluronate	47.7	3	16

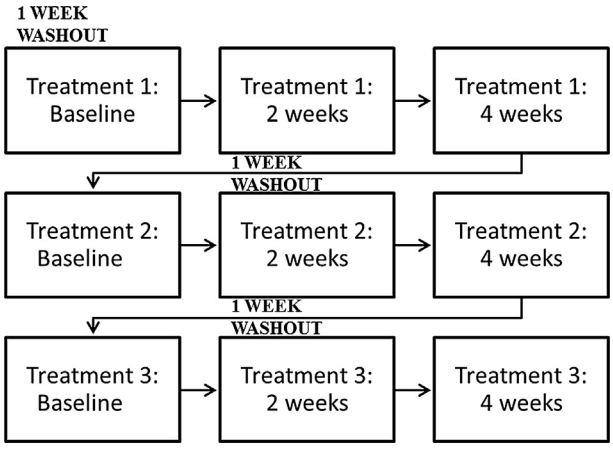

Fig. 1. Repeated measures study design as performed. Each subject completed this program of visits with a 1 week washout before starting treatment 1 and a 1 week washout between treatments.

The tear formulations tested are listed in Table 2 and will be referred to by abbreviations, for convenience. CGC (Optive Plus™, Allergan) contains the polymer carboxymethylcellulose plus glycerine and castor oil in an aqueous emulsion. It also includes the protective osmoprotectants I-carnitine and erythritol. HPG (Systane Balance®, Alcon) contains propylene glycol, hydroxypropyl guar, and mineral oil in an aqueous emulsion. CMC (Refresh Contacts®, Allergan) is an aqueous solution containing carboxymethylcellulose without any added lipid or additional non-electrolytes. All three are indicated for the relief of symptoms of dry eye conditions.

Ethical approval was granted through GCU's human subjects' ethics committee (Biomedical and Vision Sciences sub-committee). Prior to participation, written informed consent was obtained from each subject after a full explanation of the procedures involved. The study was carried out in accordance with the principles detailed in the Declaration of Helsinki.

The following assessments were carried out at each test visit:

Table 2
Details of the 3 tear formulations, including the abbreviations used.

Name	Manufacturer	Key ingredients	Preservative	Abbreviation
Optive Plus™	Allergan, plc	Carboxymethylcellulose, glycerine, castor oil, L-carnitine, erythritol	Purite®	CGC
Refresh Contacts®	Allergan, plc	Carboxymethylcellulose	Purite®	CMC
Systane Balance®	Alcon, USA	Propylene glycol, hydroxypropyl guar, mineral oil	Polyquad®	HPG

2.1. OSDI questionnaire

The OSDI questionnaire consists of 12 questions grouped into 3 sections: ocular symptoms, vision-related function and environmental factors [48]. It is designed to assess the patient's symptoms, and the impact these symptoms have on day-to-day life [48]. This questionnaire has been accepted for use in U.S. Food and Drug Administration (FDA) clinical trials for DED [48–50].

2.2. Tear osmolarity

The Ocusense TearLab Osmometer (TearLab Corporation, USA) was used to measure tear film osmolarity. Quality control (QC) was carried out each day that the TearLab was in use, as recommended by the manufacturers. The TearLab requires a tear sample of 50 nL, which is collected directly by the test card. A new single-use, sterile test card was used for each eye of every subject, at each visit. The tip of the test card was placed against the inferior temporal tear meniscus, near the outer canthus, and the sample drawn up by passive capillary action.

2.3. NITBUT

The Hir-Cal grid was mounted in a Bausch and Lomb keratometer, in which the mires had been removed. The gridlines were projected onto the ocular surface and viewed through the keratometer eyepiece, with the room lights switched off [51]. The time, in seconds, from a blink, to the point when the tear film showed any sign of breaking up, was measured [52].

2.4. Tear fluid cytokine analysis (inflammatory biomarker analysis)

A 1 μl tear sample was taken, from the study eye as determined at the first visit, from each individual using 1 disposable, sterile, micro-capillary tube (Drummond Scientific, USA). This small sample has previously been shown to be sufficient for cytokine analysis [39]. Tear fluid was collected from the tear prism situated at the outer canthus area of the eye. This region was chosen as it is a point of tear pooling, thus allowing for quicker sample collection. Great care was taken to minimize contact with the ocular surface, in order to avoid reflex tearing, as the aim was to collect samples of basal (not reflex) tears. The tear samples were centrifuged with sample diluent and stored at −80 °C until analysis.

Cytokine analysis was carried out to determine the presence and concentration of a panel of inflammatory biomarkers in the tears that have been detected in 1 μl samples [53] (Interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-17, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α)), using a multiplex immunoassay (Bio-Plex Pro Human Cytokine Multiplex Assay, Bio-Rad, UK). As this is, to our knowledge, the first time tear supplements and a panel of biomarkers have been examined in this way, and due to the inter-subject variability in the concentrations, a decrease of > 25% in inflammatory biomarker concentration has been reported as a positive response to treatment. This value was determined from previous research using anti-inflammatory formulations, where a change of as little as 22% in biomarker concentration showed statistical significance, hence this criteria was applied to the current study [40].

2.5. Tear evaporation rate (TER)

TER was measured using temperature and humidity sensors mounted in one eye-piece of a swimming goggle and linked to a ServoMed EP-3 Evaporimeter (Servo Med, Sweden) [54]. Two measurements, each lasting 2 min, were taken, one with the eye closed and one with the eye open, in order to account for evaporation from the skin of the surrounding adnexa within the goggle.

2.6. Corneal staining

The ocular surface was assessed following instillation of a small amount of sodium fluorescein from a sterile fluorescein impregnated strip (Mid-Optic, UK). Assessment was performed using a slit lamp with a cobalt blue filter and a Wratten 12 barrier filter. This allowed for the clearest view of areas of fluorescence [21]. Grading was determined using the Oxford scale (Grade 0–5), indicating the level of staining of the ocular surface [55].

2.7. Statistical analysis

All statistical analysis was carried out using the SPSS Version 21 (SPSS Inc. USA) software package. Descriptive statistics to establish mean results and standard deviations were performed. Distribution of the data was assessed using the Kolmogorov-Smirnov test. Repeated measures analysis of variance (ANOVA) and the Friedman's test were used, depending on normality, to compare the effects of the 3 treatments. Appropriate parametric and non-parametric paired samples t-tests were used to compare pre and post treatment data.

3. Results

A significant improvement (decrease) in the OSDI symptom scores was noted after 2 weeks of treatment for both the CMC and HPG treatments ($z(17) = 2.7$, $p = 0.004$ and $t(17) = 4.5$, $p < 0.001$, respectively, Fig. 2). Following 4 weeks of treatment, the improvement in OSDI scores was found to be significant for all treatments: CGC ($t(17) = 3.0$, $p = 0.008$), CMC ($z(17) = 3.1$, $p = 0.001$) and HPG ($t(17) = 4.9$, $p < 0.001$), see Fig. 2. There was no statistically significant difference between the treatments.

There was a significant improvement in NITBUT for all 3 treatments at both 2 weeks and 4 weeks (Fig. 3): CGC 2 weeks ($t(17) = 6.2$, $p < 0.001$), CGC 4 weeks ($t(17) = 5.1$, $p < 0.001$), CMC 2 weeks ($t(17) = 3.5$, $p = 0.003$), CMC 4 weeks ($t(17) = 5.7$, $p < 0.001$), HPG 2 weeks ($t(17) = 5.8$, $p < 0.001$) and HPG 4 weeks ($t(17) = 5.1$, $p < 0.001$). In the case of NITBUT, an increase in the measurement is the desired outcome as this indicates increased stability of the tear film. There was no statistically significant difference between the treatments.

Measurements of TER, tear osmolarity and corneal staining showed a reduction after 4 weeks for all 3 treatments, however this did not reach statistical significance (Table 3). A greater reduction in TER was seen with both the lipid containing-drops compared to the non-lipid containing drop. The reduction was greatest for the HPG drop ($-11.8\ g/m^2/h$), followed by the CGC drops ($-9.1\ g/m^2/h$) and, finally, the CMC drop ($-3.3\ g/m^2/h$). At the outset of this study, the variance in the TER data was unknown. Following the study, a power calculation was used to determine the number of subjects needed in

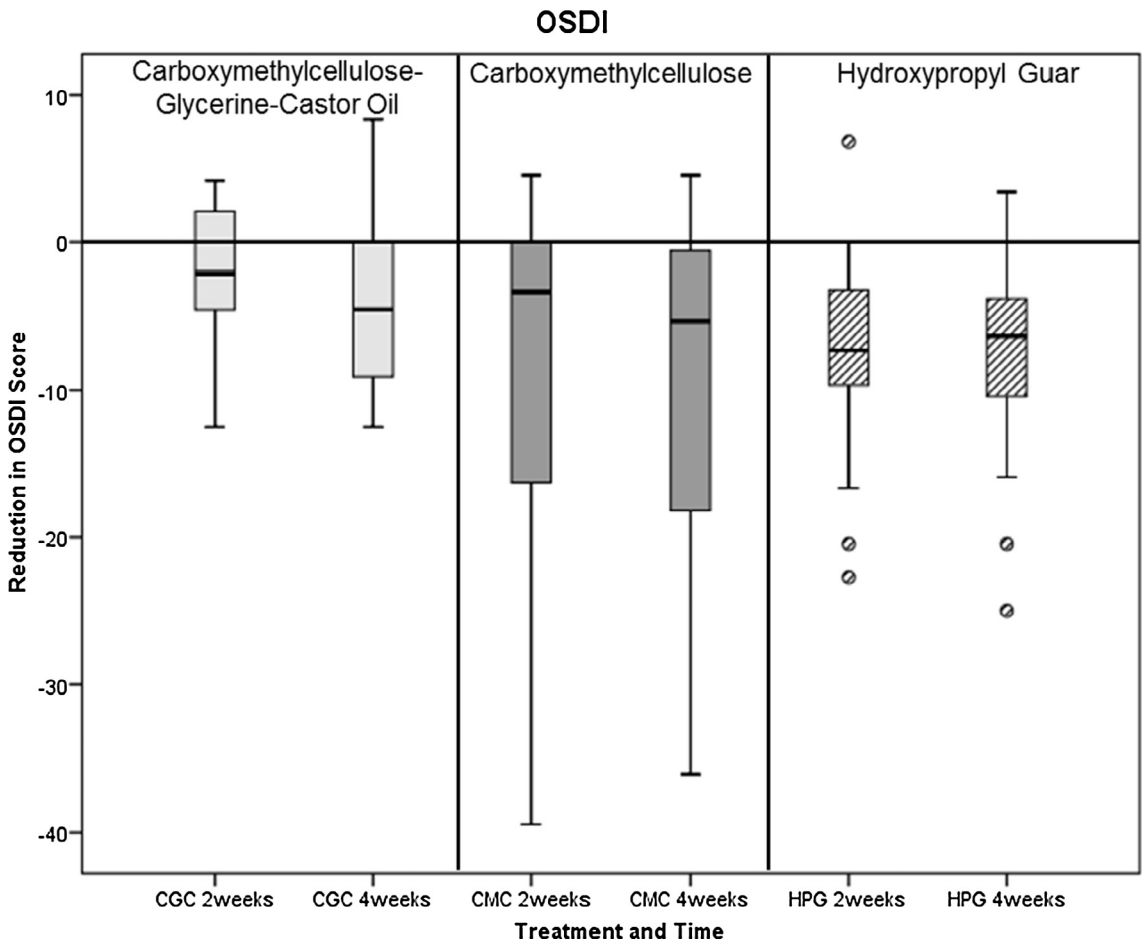

Fig. 2. Change to OSDI score with treatment. Note the zero line is baseline before treatment (probable outliers are represented by the dots) and each box represents the change from baseline to either 2 or 4 weeks (as shown on the x axis). Key: CGC = carboxymethylcellulose-glycerine-castor oil, CMC = carboxymethylcellulose, HPG = hydroxypropyl guar.

order for any change found to reach statistical significance. The result of this calculation suggests the need to recruit close to 50, rather than the 18 presented here.

Table 4 shows the baseline cytokine data for each subject (Visit 1). The average concentrations of the tear fluid inflammatory biomarkers (pg/ml) were found to be higher in this study than was the case in other, similar, studies using immunoassay kits from different manufacturers [28,30]. This may be due to the small volume collected and therefore the reduced likelihood of reflex tearing diluting the cytokine concentrations and/or the different manufacturers of the assay kits. Table 4 also highlights the inherent variability seen with this technique (and this patient demographic), as shown by the high SEM values.

Figs. 4 and 5 show the percentage of the subjects who responded to treatment for each biomarker after 2 and 4 weeks of treatment, respectively. A decrease in cytokine concentration (pg/ml) from baseline of > 25% was deemed a positive response to therapy, as previous research has shown a change of 22% can be significant [40]. After 2 weeks of treatment, the number of subjects exhibiting improvement while using CGC and CMC were similar, however fewer subjects showed this benefit when treated with HPG (Fig. 4). After 4 weeks, the CGC treatment was found to demonstrate a greater positive response rate to therapy than either of the other two drops (Fig. 5). There was no statistically significant difference between the 3 arms of the study when analyzing the percentage change of the biomarkers.

4. Discussion

In the present investigation, the CGC treatment demonstrated the greatest apparent trend for reducing levels of inflammatory biomarkers. At 4 weeks post-CGC, 6 out of 7 biomarkers demonstrated a positive response to treatment (in 40% of subjects). The same positive response was seen in 10% of the subjects for CMC and in none of the subjects for HPG. This is consistent with other findings, indicating that formulations such as CGC (which contain osmoprotectant compounds) may provide significant reductions in hyperosmolarity-induced cellular stress levels in cell and animal models (as previously measured by MAP Kinase signaling molecules, MMPs, and pro-inflammatory cytokine levels) [46,56,57].

Previous research has indicated that there is no single treatment of DED that is appropriate for all patients, due to the mixed etiology of the condition [22,58–60]. Therefore, it seemed logical to analyze the numbers of subjects who improved with each treatment. Responder analysis has been used throughout vision research to demonstrate a treatment effect in conditions such as amblyopia, glaucoma and allergic eye disease, and a responder index has been proposed for Sjögren Syndrome [61–64]. Assessing the data in terms of responders versus non-responders was considered more appropriate than using cytokine concentration data from all subjects, due to large inter-subject variations in concentrations (see Table 4) and due to variability observed in previous literature [30,65]. Additionally, biomarker percentage change was used as, to date, the biomarker levels that indicate DED are not known. Nor can it be certain that a subject has a “normal” biomarker level at the outset, as this level still needs to be established [25]. Therefore, determining if the subject had improved, compared to their own baseline level, was deemed the most appropriate method to assess treatment efficacy.

As detailed earlier, a recent report compared the effect of two artificial tear formulations on MMP-9 and IL-6 levels in humans and

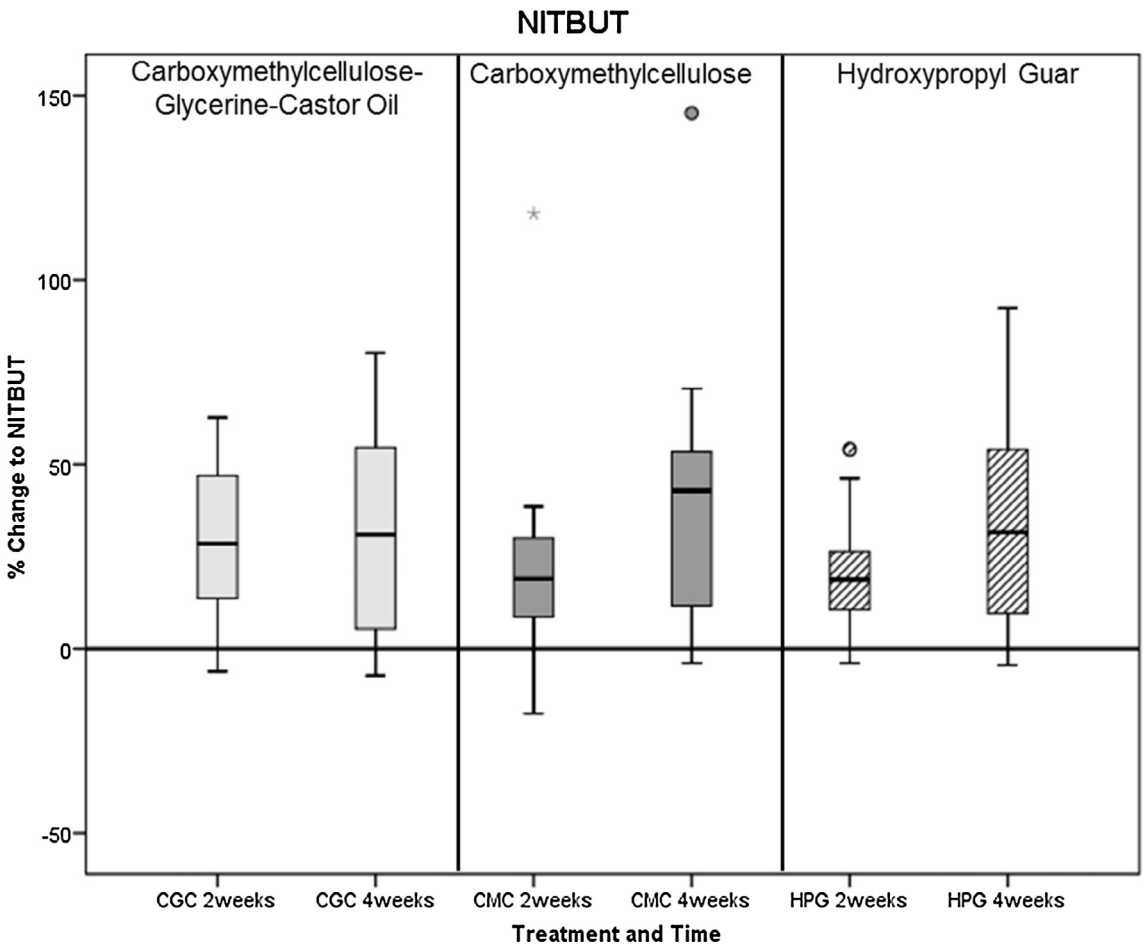

Fig. 3. Percentage change to NITBUT with treatment. Note the zero line is baseline before treatment (probable outliers are represented by the asterisk and dots) and each box represents the change from baseline to either 2 or 4 weeks (as shown on the x axis). Key: CGC = carboxymethylcellulose-glycerine-castor oil, CMC = carboxymethylcellulose, HPG = hydroxypropyl guar.

Table 3
Mean figures and standard error of mean (SEM) for the clinical parameters measured at baseline, 2 weeks and 4 weeks. All results were non-significant ($p > 0.05$) when comparing baseline with 2 and 4 weeks.

	Treatment time	CGC Mean (SEM)	CMC Mean (SEM)	HPG Mean (SEM)
TER	Baseline	82.5 (9.1)	79.5 (6.8)	77.3 (7.2)
	2 weeks	77.2 (9.7)	80.8 (10.6)	65.4 (10.3)
	4 weeks	73.4 (7.8)	76.2 (10.2)	64.8 (8.5)
Tear osmolarity	Baseline	305.3 (2.5)	304.8 (2.4)	307.4 (2.1)
	2 weeks	306.1 (2.3)	304.4 (2.7)	305.4 (2.6)
	4 weeks	303.6 (2.1)	301.8 (2.4)	302.9 (2.9)
Corneal staining	Baseline	1.3 (0.3)	1.5 (0.3)	1.5 (0.2)
	2 weeks	0.9 (0.3)	1.1 (0.2)	0.9 (0.2)
	4 weeks	1.0 (0.2)	1.0 (0.2)	0.9 (0.2)

Table 4
Cytokine values at baseline (Visit 1) for each subject in picograms per milliliter (pg/ml), including the average and SEM values.

Subject	IL-1β	IL-2	IL-6	IL-8	IL-17	IFN-γ	TNF-α
1	163	234	548	709	1299	9583	4965
2	1778	1711	2804	2029	13,139	83,388	295,850
3	67	115	233	525	3	7236	1718
4	124	156	309	503	3	7236	3119
5	391	362	716	826	1382	17,823	6511
6	3498	6053	9841	9351	25,067	77,123	58,609
7	2562	4242	7658	7887	9309	45,396	28,047
8	4426	6889	11,702	11,741	27,081	96,921	66,260
9	4017	6671	11,702	14,022	37,354	88,653	69,174
10	808	702	1539	1403	7704	39,767	26,131
11	867	1032	1980	1249	8458	39,767	28,359
12	636	712	889	927	3338	18,895	12,385
13	674	723	1421	1026	6293	30,643	20,254
14	187	168	449	477	1438	6516	2399
15	69	71	197	348	3	1929	1096
16	164	168	380	550	1353	12,189	5217
17	466	395	672	627	5726	21,921	9281
18	339	268	628	477	2947	15,159	6557
Mean	1180	1704	2981	3038	8439	34,452	21,093
SEM	341	573	971	1039	2534	7394	5288

found that an aqueous formulation with carboxymethylcellulose and osmoprotectants reduced the level of inflammation [44]. However, the study assessed MMP-9 and IL-6 staining from impression cytology, which is a less quantitative method of assessing inflammation, and examined just 2 pro-inflammatory molecules. If cellular stress is reduced by artificial tear treatment, it could be expected that the stimulus for pro-inflammatory signaling and the subsequent production of inflammatory biomarkers may be lower. There may be explanations, other than there being a direct benefit from the treatment, for the reduction in inflammatory biomarkers observed in this study. For example, interaction between any one of the components of the CGC formulation might affect detection of the inflammatory biomarkers in the tears. Alternatively, interference with the multiplex assay technique itself, resulting in an apparent reduction in cytokine levels, could also have occurred. Since there was a minimum time period of 2 h between

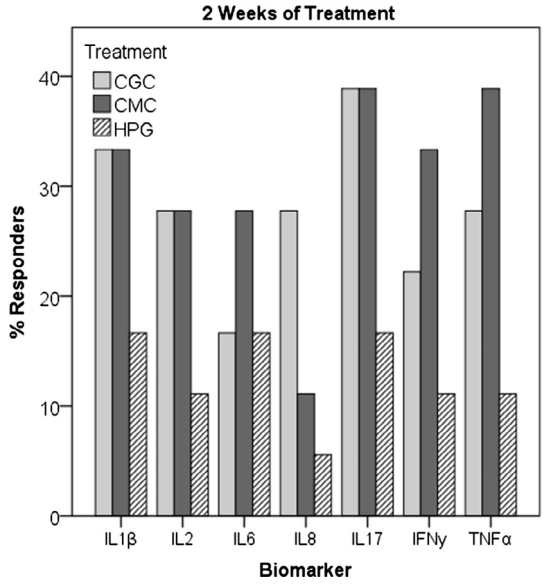

Fig. 4. Percentage "responders" (decrease in biomarker concentration > 25%) for each treatment after 2 weeks of eye drop use. Key: CGC = carboxymethylcellulose-glycerine-castor oil, CMC = carboxymethylcellulose, HPG = hydroxypropyl guar.

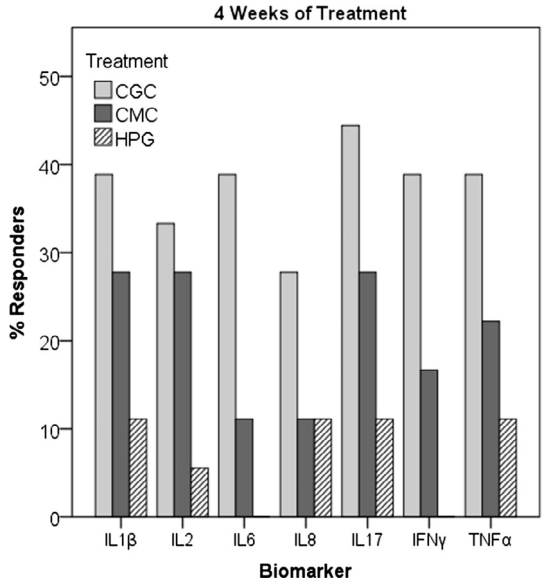

Fig. 5. Percentage "responders" (decrease in biomarker concentration > 25%) for each treatment after 4 weeks of eye drop use. Key: CGC = carboxymethylcellulose-glycerine-castor oil, CMC = carboxymethylcellulose, HPG = hydroxypropyl guar.

the use of the drops and tear sampling, such interference is unlikely, due to the tear turnover rate [47,66]. Castor oil, however, has been reported to remain on the ocular surface up to 4 h after instillation [67]. Therefore, further research investigating the residency time of the various components of these eye drops would be beneficial.

A significant improvement in symptoms and NITBUT was observed for all 3 treatments; however this was not associated with a significant improvement in the measures of osmolarity, TER and corneal staining, or in the inflammatory biomarker levels. It is possible that there is a time lag between the initial symptom relief and improvement in NITBUT, following regular use of the eye drops, and a corresponding change to the underlying disease mechanism. As shown in Fig. 2, all three treatments resulted in a worsening of symptoms for some of the subjects. This was not surprising, as there is no consensus on which artificial tear is the most effective in managing signs and symptoms and it is anecdotally known that different patients with DED prefer different drops [68].

Previous research using the anti-inflammatory formulations cyclosporine A and methylprednisolone has shown a significant improvement in inflammatory biomarker levels in the conjunctiva, after 3 months of treatment [69]. While our current study also demonstrated *reduced* inflammatory biomarkers in response to treatment, it is possible that a longer treatment period is required to achieve a statistically significant effect on cytokine levels with artificial tears. The apparent reduction in biomarker levels after 2–4 weeks of treatment does suggest, however, that osmoprotectants have a more rapidly-acting mechanism than anti-inflammatory medications. For example, a rapid response has been shown for over-the-counter tear formulations after only 2 weeks treatment, in a murine model of dry eye (for corneal staining and goblet cell density) [57]. Moreover, a longer treatment period (3 months) has shown statistically significant reductions in clinical measures such as TER, osmolarity and corneal staining with artificial tears [70]. It may be that over a longer time course, a significant reduction will also be seen in the inflammatory biomarkers. Further research, following these parameters for a longer period of time, e.g. 3 months, is therefore necessary. This would facilitate examination of the time course of the inflammatory biomarkers during treatment.

It should be noted that all of these drops contained preservatives, although the preservatives varied. The CGC and CMC drops contained Purite®, a stabilized oxychloro complex (SOC), which breaks down into natural tear components (water, oxygen, sodium and chloride ions) on contact with the ocular surface [71]. In animal studies, Purite® has been shown to cause minimal epithelial erosion, when compared to Polyquad® (the preservative in the HPG formulation), which is a detergent-type preservative that may cause corneal epithelial erosion [71]. It is possible that Polyquad® contributed to the poorer response of the HPG formulation, as it has been shown, in some studies, to induce an inflammatory reaction in cultured human corneal cells [72]. Conversely, other research found the opposite effect with Polyquad® showing good tolerability on human corneal cells [73]. Another study in an animal wound-healing model has suggested that Purite-preserved eye drops may have some deleterious effects on the ocular surface [74], and in a clinical study of Purite-preserved drops vs a similar formulation without preservative, small differences were detected in favor of the non-preserved drop [75]. It is certainly possible that some of the results of the present study were influenced by the presence of the preservative. However, due to the inconclusive results of previous research and lack of dry eye studies, further work investigating these preservatives *in vivo* would be valuable [76].

It is also important to consider the inclusion criteria (NITBUT < 10 s and OSDI > 12) employed in this study, as the average NITBUT of the subjects at baseline was 5.7 (± 1.2) s and the OSDI was 23 (± 12.8). The NITBUT indicates that the subjects enrolled in the study were generally of mild/moderate disease severity [10,77]. Therefore, it should be considered how likely a significant improvement, in both clinical and cytokine measures would be. In other words, is there scope for significant improvements, when the disease is of the mild/moderate severity? Moreover, as NITBUT was used as a clinical measure for inclusion, it is likely the subjects' DED may be more evaporative in nature. Several different clinical variables (as well as age, sex and treatment order) were examined to see if patterns were present in those who responded to the CGC treatment. One possible link related to the tear production, as measured by the Schirmer test. This measure was, generally lower amongst CGC responders, with an average of 9 mm, versus 14 mm for those who did not respond indicating that the responders were more aqueous deficient in nature. It should be noted that 10 mm of wetting is a commonly used upper limit for aqueous deficient dry eye [25,78]. This combination of different signs and symptoms is reflective of what commonly presents to ophthalmic clinicians and directly relates to the complex etiology of DED. This suggests that further research investigating the different sub-groups of DED would help to improve the treatments currently used in practice to

reduce symptoms and levels of inflammation. It must also be acknowledged that this was a relatively young DED subject group (30 ± 14 years) and therefore a similar study on a group of older subjects, where DED is more prevalent, would be of significant interest.

5. Conclusions

In conclusion, of the 3 products tested, the eyedrop containing a polymeric lubricant, emulsified lipid and osmoprotective non-electrolytes appeared to be the most effective in reducing DED-associated tear film inflammatory biomarkers. This finding is supportive of the concept that improving the ocular surface environment is likely to reduce ocular pro-inflammatory cytokine activation.

Conflict of interest

Author Peter Simmons was an employee of Allergan plc who manufacture two of the test products.

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